

WHAT IS CLAIMED IS:

- 1           1. An isolated infectious chimeric parainfluenza virus (PIV) comprising a  
2 major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein  
3 (L), and a human PIV (HPIV) vector genome or antigenome that is modified to encode a  
4 chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments,  
5 or epitopes of a second, antigenically distinct HPIV.
- 1           2. The chimeric PIV of claim 1, wherein one or more heterologous genome  
2 segment(s) of the second, antigenically distinct HPIV encoding said one or more antigenic  
3 domains, fragments, or epitopes is/are substituted within the HPIV vector genome or  
4 antigenome to encode said chimeric glycoprotein.
- 1           3. The chimeric PIV of claim 2, wherein said one or more heterologous  
2 genome segment(s) encode(s) one or more glycoprotein ectodomain(s) substituted for one or  
3 more corresponding glycoprotein ectodomain(s) in the vector genome or antigenome.
- 1           4. The chimeric PIV of claim 2, wherein heterologous genome segments  
2 encoding both a glycoprotein ectodomain and transmembrane region are substituted for  
3 counterpart glycoprotein ecto- and transmembrane domains in the vector genome or  
4 antigenome.
- 1           5. The chimeric PIV of claim 1, wherein said chimeric glycoprotein is  
2 selected from HPIV HN or F glycoproteins.
- 1           6. The chimeric PIV of claim 1, wherein the (HPIV) vector genome or  
2 antigenome is modified to encode multiple chimeric glycoproteins.
- 1           7. The chimeric PIV of claim 1, wherein the HPIV vector genome or  
2 antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct  
3 HPIV is selected from HPIV1 or HPIV2.
- 1           8. The chimeric PIV of claim 7, wherein the HPIV vector genome or  
2 antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct  
3 HPIV is HPIV2.

1           9.     The chimeric PIV of claim 8, wherein one or more glycoprotein  
2     ectodomain(s) of HPIV2 is/are substituted for one or more corresponding glycoprotein  
3     ectodomain(s) in the HPIV3 vector genome or antigenome.

1           10.    The chimeric PIV of claim 9, wherein both glycoprotein ectodomain(s)  
2     of HPIV2 HN and F glycoproteins are substituted for corresponding HN and F glycoprotein  
3     ectodomains in the HPIV3 vector genome or antigenome.

1           11.    The chimeric PIV of claim 10, which is rPIV3-2TM.

1           12.    The chimeric PIV of claim 10, which is further modified to incorporate  
2     one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp45*.

1           13.    The chimeric PIV of claim 12, which is rPIV3-2TM*cp45*

1           14.    The chimeric PIV of claim 8, wherein PIV2 ectodomain and  
2     transmembrane regions of one or both HN and/or F glycoproteins is/are fused to one or more  
3     corresponding PIV3 cytoplasmic tail region(s).

1           15.    The chimeric PIV of claim 14, wherein ectodomain and transmembrane  
2     regions of both PIV2 HN and F glycoproteins are fused to corresponding PIV3 HN and F  
3     cytoplasmic tail regions.

1           16.    The chimeric PIV of claim 15, which is rPIV3-2CT.

1           17.    The chimeric PIV of claim 16, which is further modified to incorporate  
2     one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp45*.

1           18.    The chimeric PIV of claim 15, which is rPIV3-2CT*cp45*.

1           19.    The chimeric PIV of claim 1, which is further modified to incorporate  
2     one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp45*  
3     selected from mutations specifying an amino acid substitution in the L protein at a position  
4     corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in the N protein at a position  
5     corresponding to residues Val96 or Ser389 of JS *cp45*, in the C protein at a position  
6     corresponding to Ile96 of JS *cp45*, a nucleotide substitution in a 3' leader sequence of the  
7     chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or a  
8     mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS *cp45*

1           20. The chimeric PIV of claim 1, wherein a plurality of heterologous genes  
2 or genome segments encoding antigenic determinants of multiple heterologous PIVs are added  
3 to or incorporated within the partial or complete HPIV vector genome or antigenome.

1           21. The chimeric PIV of claim 20, wherein said plurality of heterologous  
2 genes or genome segments encode antigenic determinants from both HPIV1 and HPIV2 and  
3 are added to or incorporated within a partial or complete HPIV3 vector genome or antigenome.

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1           22. The chimeric PIV of claim 20, wherein the chimeric genome or  
2 antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or  
3 epitopes from two or more different HPIVs.

1           23. The chimeric PIV of claim 1, wherein the chimeric PIV genome or  
2 antigenome is attenuated by addition or incorporation of one gene or cis-acting regulatory  
3 element from a bovine PIV3 (BPIV3).

1           24. The chimeric PIV of claim 1, wherein the chimeric PIV genome or  
2 antigenome incorporates one or more heterologous, non-coding non-sense polynucleotide  
3 sequence(s).

1           25. The chimeric PIV of claim 1, wherein the chimeric genome or  
2 antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or  
3 epitopes from both HPIV3 JS and HPIV1 or HPIV2.

1           26. The chimeric PIV of claim 1, wherein the chimeric genome or  
2 antigenome is modified by introduction of an attenuating mutation involving an amino  
3 acid substitution of phenylalanine at position 456 of the HPIV3 L protein.

1           27. The chimeric PIV of claim 26, wherein phenylalanine at position  
2 456 of the HPIV3 L protein is substituted by leucine.

1           28. The chimeric PIV of claim 1, wherein the chimeric genome or  
2 antigenome incorporates one or more heterologous gene(s) or genome segment(s)  
3 encoding one or more respiratory syncytial virus (RSV) F and/or G glycoprotein(s) or  
4 immunogenic domain(s), fragment(s), or epitope(s) thereof.

1           29. The chimeric PIV of claim 1 which is a virus.  
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1           30. The chimeric PIV of claim 1 which is a subviral particle.

1           31. A method for stimulating the immune system of an individual to induce  
2 protection against PIV which comprises administering to the individual an immunologically  
3 sufficient amount of the chimeric PIV of claim 1 combined with a physiologically acceptable  
4 carrier.

1           32. The method of claim 31, wherein the chimeric PIV is administered in a  
2 dose of  $10^3$  to  $10^7$  PFU.

1           33. The method of claim 31, wherein the chimeric PIV is administered to  
2 the upper respiratory tract.

1           34. The method of claim 31, wherein the chimeric PIV is administered by  
2 spray, droplet or aerosol.

1           35. The method of claim 31, wherein the vector genome or antigenome is of  
2 human PIV3 (HPIV3) and the chimeric PIV elicits an immune response against HPIV1 and/or  
3 HPIV2.

1           36. The method of claim 31, wherein the chimeric PIV elicits a polyspecific  
2 immune response against multiple human PIVs.

1           37. The method of claim 31, wherein a first, chimeric PIV and a second PIV  
2 are administered sequentially or simultaneously to elicit a polyspecific immune response.

1           38. The method of claim 37, wherein the second PIV is a second, chimeric .  
2 PIV according to claim 1.

1           39. The method of claim 37, wherein the first, chimeric PIV and second PIV  
2 are administered simultaneously in a mixture.

1           40. The method of claim 37, wherein the first and second chimeric PIVs are  
2 bear the same or different heterologous antigenic determinant(s).

1           41. The method of claim 37, wherein the first chimeric PIV elicits an  
2 immune response against HPIV3 and the second chimeric PIV elicits an immune response  
3 against HPIV1 or HPIV2.

1                  42. The method of claim 37, wherein the second chimeric PIV incorporates  
2 one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic  
3 determinant(s) of respiratory syncytial virus (RSV).

1                  43. The method of claim 42, wherein both the first and second chimeric  
2 PIVs elicit an immune response against RSV.

1                  44. The method of claim 43, wherein the first chimeric PIV is administered  
2 initially in a vaccination protocol and the second chimeric PIV is administered subsequently in  
3 the vaccination protocol to provide initial immunization against HPIV3 and secondary  
4 immunization against HPIV1 or HPIV2 and to provide initial and secondary, booster  
5 immunization against RSV.

1                  45 . The method of claim 37, wherein the first, chimeric PIV incorporates at  
2 least one and up to a full complement of attenuating mutations present within PIV3 JS cp45  
3 selected from mutations specifying an amino acid substitution in the L protein at a position  
4 corresponding to Tyr942, Leu992, or Thr1558 of JS cp45; in the N protein at a position  
5 corresponding to residues Val96 or Ser389 of JS cp45, in the C protein at a position  
6 corresponding to Ile96 of JS cp45, a nucleotide substitution in a 3' leader sequence of the  
7 chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS cp45, and/or a  
8 mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS cp45.

1                  46. An immunogenic composition to elicit an immune response against PIV  
2 comprising an immunogenically sufficient amount of the chimeric PIV of claim 1 in a  
3 physiologically acceptable carrier.

1                  47. The immunogenic composition of claim 46, formulated in a dose of  $10^3$   
2 to  $10^7$  PFU.

1                  48. The immunogenic composition of claim 46, formulated for  
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1                  49. The immunogenic composition of claim 46, wherein the chimeric PIV  
2 elicits an immune response against one or more virus(es) selected from HPIV1, HPIV2 and  
3 HPIV3.

1               50.     The immunogenic composition of claim 46, wherein the chimeric PIV  
2     elicits an immune response against HPIV3 and another virus selected from HPIV1, HPIV2,  
3     and respiratory syncytial virus (RSV).

1               51.     The immunogenic composition of claim 46, further comprising a  
2     second, chimeric PIV according to claim 1.

1               52.     The immunogenic composition of claim 51, wherein the first chimeric  
2     PIV elicits an immune response against HPIV3 and the second chimeric PIV elicits an immune  
3     response against HPIV1 or HPIV2, and wherein both the first and second chimeric PIVs elicit  
4     an immune response against RSV.

1               53.     An isolated polynucleotide comprising a chimeric PIV genome or  
2     antigenome which includes a human PIV (HPIV) vector genome or antigenome modified to  
3     encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains,  
4     fragments, or epitopes of a second, antigenically distinct HPIV.

1               54.     The isolated polynucleotide of claim 53, wherein one or more  
2     heterologous genome segment(s) encoding the antigenic domains, fragments, or epitopes of  
3     said second, antigenically distinct HPIV is/are substituted for one or more counterpart genome  
4     segment(s) in the HPIV vector genome or antigenome.

1               55.     The isolated polynucleotide of claim 53, wherein, the chimeric genome  
2     or antigenome incorporates at least one and up to a full complement of attenuating mutations  
3     present within PIV3 JS *cp45*.

1               56.     A method for producing an infectious attenuated chimeric PIV particle  
2     from one or more isolated polynucleotide molecules encoding said PIV, comprising:

3               expressing in a cell or cell-free lysate an expression vector comprising an  
4     isolated polynucleotide comprising a vector genome or antigenome modified to encode a  
5     chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments,  
6     or epitopes of a second, antigenically distinct HPIV, and PIV N, P, and L proteins.

1               57.     The method of claim 56, wherein the chimeric PIV genome or  
2     antigenome and the N, P, and L proteins are expressed by two or more different expression  
3     vectors.

1           58. An expression vector comprising an operably linked transcriptional  
2 promoter, a polynucleotide sequence which includes a vector genome or antigenome modified  
3 to encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains,  
4 fragments, or epitopes of a second, antigenically distinct HPIV, and a transcriptional  
5 terminator.